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# Intestinal Rotation and Physiological Umbilical Herniation During the Embryonic Period

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**Intestinal rotation and physiological umbilical herniation during the embryonic period**

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## 26    **Abstract**

27    Drastic changes occur during the formation of the intestinal loop (IL), including  
28    elongation, physiological umbilical herniation (PUH), and midgut rotation.  
29    Fifty-four sets of magnetic resonance images of embryos between Carnegie  
30    stage (CS) 14 and CS 23 were used to reconstruct embryonic digestive tract in  
31    three dimensions in the Amira program. Elongation, PUH, and rotation were  
32    quantified in relation to the proximal part of the superior mesenteric artery (SMA),  
33    designated as the origin. Up to CS 16, IL rotation was initially observed as a  
34    slight deviation of the duodenum and colorectum from the median plane. The  
35    PUH was noticeable after CS 17. At CS 18, the IL showed a hairpin-like structure,  
36    with the SMA running parallel to the straight part and the cecum located to the  
37    left. After CS 19, the IL began to form a complex structure as a result of the rapid  
38    growth of the small intestinal portion. By CS 20, the IL starting point had moved  
39    from the right cranial region to an area caudal to the origin, though elongation of  
40    the duodenum was not conspicuous—this was a change of almost 180° in  
41    position. The end of the IL remained in roughly the same place, to the left of and  
42    caudal to the origin. Notably, the IL rotated around the origin only during earlier  
43    stages and gradually moved away, running transversely after CS 19. The  
44    movements of the IL may be explained as the result of differential growth,  
45    suggesting that IL rotation is passive.

46



47    **Key Words**

48    human embryo, intestinal development, midgut rotation, magnetic resonance  
49    imaging, three-dimensional reconstruction

50    **Abbreviation**

51    superior mesenteric artery (SMA), intestinal loop (IL), OE; omphalo-enteric duct,  
52    magnetic resonance (MR), Carnegie stage (CS), Three-dimensional (3D),  
53    Starting point of IL (S), Ending point of IL (E), Crown-Ramp length (CRL)  
54    point near the pyloric antrum (P), starting of IL (S), ending of IL, (E), cecum (C),  
55    tip of the IL (T), point crossing the border between abdominal coelom and  
56    extraembryonic coelom in the umbilical cord on the colon (B), omphalo-enteric  
57    duct (OE), angle between median plane and segment SE was calculated ( $\angle Ro$ )

## 58 Introduction

59       The intestine elongates considerably during the embryonic period, and this  
60 involves two important phenomena, physiological umbilical herniation (PUH)  
61 (Meckel, 1817) and rotation (Mall, 1898). Those phenomena occur  
62 simultaneously and affect each other, making the process complicated to  
63 describe. Briefly, in PUH, the elongated intestine temporarily enters the  
64 extraembryonic coelom in the umbilical cord, rotating through 90° in a  
65 counterclockwise direction. This is followed by reduction, in which the intestine  
66 returns to the abdominal coelom, rotating a further 90° counterclockwise. In the  
67 final phase, fixation, the intestine assumes its final position as the mesentery  
68 rotates through 90°, again counterclockwise. The timing of PUH (Frazer and  
69 Robbins, 1915; O’Rahilly and Muller, 2001; Cyr et al., 1986) and the speed and  
70 timing of reduction (Mall, 1898; Cyr et al., 1986, Snyder and Chaffin, 1952, 1954)  
71 were subjects of debate until Kim et al. (2003), using histological analysis, found  
72 that initiation of the intestinal loop (IL) occurs at Carnegie stage (CS) 14, with  
73 PUH beginning around CS 16 and reaching its maximum at CS 23. The IL  
74 returns to the abdominal coelom when the crown-rump length (CRL) of the fetus  
75 is around 40 mm. However, the timing and degree of rotation are still being  
76 argued (Frazer and Robbins, 1915; Dott, 1922). In fact, Kluth et al. (1995, 2003,  
77 2011) claim that there is no conclusive evidence for the rotation of the gut,  
78 because a malrotated gut has never been observed in normal embryos. On the  
79 basis of a scanning electron microscope study of IL formation in a rat model,  
80 they emphasize the importance of the formation of the duodenal loop and state  
81 that malformations are exclusively the result of localized growth failures of this

loop. They also deny that the cecum moves to the right lower part of the abdomen during fixation. Soffers et al. (2015) have developed hierarchical models based on 3D reconstruction of the midgut, superior mesenteric artery (SMA), and mesentery as a whole from histological sections; their models also differ from the classical en bloc rotation model.

Analysis and understanding of embryological intestinal development is undoubtedly important, as failure of the process can give rise to malformations such as nonrotation, malrotation, and subhepatic cecum. There is a need for quantitative, stage-by-stage descriptions of the process, based on three-dimensional (3D) images. The present study was designed to document and measure the movements of the intestine during elongation, PUH, and rotation in three dimensions; this was done using magnetic resonance (MR) microscopy to obtain images of embryos between CS 14 and CS 23, corresponding to the period from the initiation of the IL to maximum PUH.

## Materials and Methods

### *Human embryo specimens*

Approximately 44,000 human embryos, comprising the Kyoto Collection of Human Embryos, are stored at the Congenital Anomaly Research Center of Kyoto University (Nishimura et al., 1968; Shiota et al., 2007; Yamada et al., 2006). Most of these were obtained after termination of a pregnancy during the first trimester for socioeconomic reasons under the Maternity Protection Law of Japan. In the laboratory, aborted embryos were measured, examined, and

staged by two of the authors (C. U. and S. Y.) using the criteria provided by O’Rahilly and Müller (1987). Approximately 1,200 well-preserved embryos, found to be normal on gross examination and between CS 14 and CS 23, were selected for MR microscopy using methods previously described elsewhere (Shiota et al., 2007; Yamada et al., 2006; Matsuda et al., 2003, 2007). Of the 1,200 resulting MR image sets, the authors selected 54 displaying an intact umbilicus and body area for analysis of dimensional changes (3–7 for each CS).

### ***Image analysis***

The structure of the digestive tract, including the stomach, duodenum, IL, and colorectum, was reconstructed for each image set using the Amira software suite (version 5.4.5; Visage Imaging, Berlin, Germany) (Fig. 1a). The AmiraSkel software module was used to determine the centerline length of the digestive tract.

### ***Anatomical landmarks and position of the intestine***

The 3D coordinates were initially assigned by using Amira to examine the positions of particular voxels in 3D images. Two points on the proximal straight part of the SMA were designated Z1 and Z2 and used as external anatomical landmarks, and the line connecting the points was defined as the z-axis of the coordinate system (Fig. 1a,b). Point P, located on the digestive tract near the pyloric antrum above the neural tube and vertebral column, was used to determine the median plane and y-axis (Fig. 1b) (Kaigai et al., 2014). Various other points on the tract were used as internal anatomical landmarks, including

the start of the IL (S), the end of the IL (E), the cecum (C), the tip of the IL (T), and point B, located where the border between the abdominal coelom and extraembryonic coelom (specifically the umbilical cord) crossed the straight part of the colonic portion of the IL. S and E were defined as the most obvious dorsal inflection points on the 3D image and may correspond to the points at which the length of the mesentery drastically changed as described by Soffers et al. (2015).

The lengths of the duodenum (segment PS), small intestinal part of the IL (segment SC), and large intestinal part of the IL (segment CE) were measured. The distance from E to the rectum was not measured because it was difficult to precisely locate the colorectum within the pelvic cavity using our methods. The heights of T, B, and C were measured to estimate the extent of PUH (Fig. 1c). Point T corresponded to the entry of the omphaloenteric duct, which could occasionally be visualized. The z-coordinate of each landmark was recorded as its height. CRL and maximal abdominal transverse length were used as references for embryonic axial growth and width, respectively. To estimate the position and rotation of the IL at points S and E, the angle between the median plane and segment SE was calculated (angle of rotation,  $\angle Ro$ ). The angles formed with the median plane by points S and E were measured as  $\angle s$  and  $\angle e$ , respectively, and the length of segment SE was also measured (Fig.1d). The ethics committee of the Kyoto University Graduate School and Faculty of Medicine approved this study (E986).

## 153 **Results**

### 154 ***Morphological changes of the intestinal tract during the embryonic period***

155 The 3D anatomy of the intestinal tract was reconstructed using Amira (Fig.  
156 2). The IL's development of a complex coiled, spiral structure during  
157 development was successfully visualized. Elevation of the intestinal tract was  
158 already observable at CS 14 (Fig. 2a), though points S and E were not clearly  
159 defined. From CS 16 onward, points S and E and the cecum were clearly  
160 recognizable. At CS 16, point T was located near the border between the  
161 abdominal coelom and extraembryonic coelom. The border area was broad and  
162 tilted caudad, making it difficult to determine whether the tip of the intestine had  
163 entered the umbilical cord or not. Both the duodenum and colorectum ran  
164 parallel to the median plane, with the duodenum shifted slightly to the right and  
165 the colorectum shifted slightly to the left. The IL connected both parts obliquely  
166 like a bridge. Viewed ventrally, the tract formed a crank shape (Fig. 2a).

167 At CS 17, PUH of the intestine, including the cecum, was evident (data not  
168 shown). By CS 18, the intestine had elongated and formed a straight tube like a  
169 hairpin (Fig. 2b). The SMA ran straight, parallel and proximate to both the  
170 efferent and afferent parts of the IL. The efferent part was on the right of the  
171 SMA, and the afferent part was on the left. The cecum and vermiform appendix  
172 could be distinguished to the left of the SMA in the umbilicus.

173 From CS 19 onward, the small intestinal portion of the IL began to show  
174 marked elongation and coiling, whereas the large intestinal portion remained  
175 almost straight until CS 23 (Fig. 2c). At these later stages, point E was located  
176 near the left gonadal ridge and metanephros, which were caudal to the origin

177 (data not shown).

178

## 179 ***Dimensional changes of the intestinal tract from CS 14 to CS 23***

180

### 181 ***Elongation of the intestinal tract***

182 The lengths of each previously mentioned segment of the intestinal tract  
183 were measured. CRL, the indicator of embryonic axial growth, increased  
184 nearly 3.6 times greater at CS 23 than at CS 16 (Fig. 3). The intestinal tract  
185 elongated rapidly over this period, primarily owing to the growth of segment SC  
186 (the small intestinal part of the IL). As a result, looping, coiling, and spiral  
187 formation were observed, first in the umbilicus and later inside the abdominal  
188 coelom. The lengths of the duodenum and the large intestinal part of the IL  
189 increased gradually over time. Compared with CS 16, the small intestinal part of  
190 the IL was 20 times longer by CS 23, whereas the large intestinal part was 8  
191 times longer and the duodenum only 3.5 times longer.

192

### 193 ***Physiological umbilical herniation of the intestinal tract***

194 The heights of the point of PUH and the border of the abdominal coelom  
195 increased gradually, at a rate similar to the increase in CRL (Fig. 4). The height  
196 ratios between T and B and between C and B were almost constant between CS  
197 17 and CS 23 (T to B, 1.79–2.13; C to B, 1.10–1.38). The cecum could  
198 consistently be located at the proximal part of the umbilical cord, implying that  
199 the main part of the colorectum remained inside the abdominal coelom.

200

## 201 *Movements of the intestinal tract*

202       Points S and E were plotted on the coordinates with the proximal part of the  
203 SMA as the origin (Fig. 5a). As CS stage advanced, Point S moved caudally  
204 from the right cranial region of the SMA (in the second quadrant) to a point just  
205 right of the SMA, finally settling caudad of origin, almost in the median plane.  
206 Point E started out left of and caudal to the SMA (in the fourth quadrant) and  
207 gradually moved caudad. The distance between points S and E remained almost  
208 constant between CS16 and CS23 (715–1330  $\mu\text{m}$  on the xy plane). The  
209 maximum abdominal transverse length increased gradually from 2.43 mm at CS  
210 16 to 6.24 mm at CS 23.

211       Viewed ventrally, the IL was slightly tilted toward the median plane (Fig. 2a)  
212 at angle of  $16.7^\circ$  at CS 14 and  $34.5^\circ$  at CS 15. Though points S and C were  
213 clearly identifiable after CS 16, the angle between segment SE and the median  
214 plane ( $\angle\text{Ro}$ ), which was  $43.6^\circ$  at CS 16, reached near perpendicularity ( $92.3^\circ$ ) at  
215 CS 19 (Fig. 6). Notably, segment SE was rotated around the SMA only during  
216 earlier stages (up to CS 17); after CS 18, it moved caudad from the origin (Fig.  
217 5a).

218       The movements of points S and E relative to the SMA were measured by  
219 their angles to the median plane. From CS 16 to CS 17,  $\angle\text{s}$  increased from  $59.9^\circ$   
220 to  $106.8^\circ$ ; by CS 23, it was  $184.1^\circ$ . In contrast,  $\angle\text{e}$  remained almost constant  
221 from CS16 to CS23 (changing from  $25.5^\circ$  and  $44.6^\circ$ ).



The cecum was consistently located to the left of the SMA, generally at a distance of 500 to 1000  $\mu$ m, though position varied as development proceeded (Fig. 5b).

## Discussion

In the present study, we used 3D reconstruction to observe the dimensional changes of the intestinal tract between CS 14 and CS 23, corresponding to the period from the formation of the IL to PUH. Our observations were consistent with previous reports of the timeline of development (Kim et al., 2003), with the IL already observable at CS 14. PUH started at CS 16 and was noticeable by CS 17, with the extent of PUH increasing until CS 23.

Regarding rotation, there were several notable findings. The intestinal tract initially ran in the median plane, which was connected with the mesentery (Snyder and Chaffin, 1954). The transverse distance between the duodenum and colorectum changed slightly but significantly from CS 14 to CS 16, which formed a crank shape in ventral view with points S and E as vertices (Figs. 2a, 7a). Though the mechanism by which this laterality might happen is not yet known, it may be related to the developing liver and left umbilical vein (Frazer and Robbins, 1915) or to the helical body axis and descent of the upper abdominal structure (Soffers et al., 2015). The discrepancies in the literature regarding the timing of rotation initiation may result from this small but important phenomenon being overlooked or not regarded as part of rotation. Kim et al.

(2003) and Mall (1898) indicate that rotation begins at CS 15, just before the IL enters the umbilical cord. Other authors place the initiation of PUH and that of rotation at same time, around CS 16 or 17 (Frazer and Robbins, 1915).

We observed the origin (the proximal part of the SMA) to be located along segment SE at earlier stages. However, this segment gradually moved caudad, finally becoming horizontal to the body axis around CS 19, which was later by 4 stages than previously reported (Kim et al., 2003). The event seemed to be a result of the gradual movement of points S and E. Previous studies have reported the colorectum to be important for rotation (Frazer and Robbins, 1915; Dott, 1923), stating that as it becomes considerably elongated and rotates counterclockwise, it pushes the rest of the intestinal tract with it. However, movement of point E was not conspicuous in the present study; indeed, the position of point E relative to the origin was very stable, and what movement there was caudad. We observed the elongation of segment CE to be relatively slow, with the colorectum not playing a prominent role in the observed events.

On the other hand, the position of S to the origin, expressed by  $\angle s$ , changed dramatically during development, rotating nearly  $180^\circ$  during PUH, and the rate of elongation of the duodenum was similar to that of the increase in CRL. The movement of point S was caudad, almost along the medial plane. These findings suggest the movement of the start of the IL may be appropriately explained as the result of differential growth rather than rotation around the SMA.

The distance between S and E was almost constant. Maximal abdominal

269 transverse length increased by only 2.6 times from CS 16 to CS 23, so the final  
270 ratio of distance between S and E to abdominal transverse length was rather  
271 small. Point S was located almost in the median plane at CS 20, though the time  
272 at which it reached this position was earlier than previously described (Mall,  
273 1898; Frazer and Robbins, 1915; Snyder and Chaffin, 1954; Dott, 1923; Kluth et  
274 al., 1995, 2003; Metzger et al., 2011).

275 Kluth et al. (1995, 2003) and Metzger et al. (2011) recently made detailed  
276 observations of midgut morphogenesis, including PUH and the process of return,  
277 in rats between embryonic days (EDs) 13 and 17. Six of their findings were  
278 consistent with ours. First, at ED 14 (corresponding to CS 18), the cecum was  
279 located to the left of the SMA as the result of the elongation of the small intestine  
280 in the umbilicus. Second, when the intestine entered the umbilical cord at ED 15  
281 (corresponding to CS 20), the tip of the duodenojejunal area was already located  
282 beneath the root of the mesentery. Third, at ED 15, the position of the cecum  
283 varied between embryos. Fourth, at ED 15, the colorectum was mainly located in  
284 the abdominal coelom. Fifth, the active contribution of the colorectum to the  
285 development of the IL was small throughout the observation period. Sixth, the  
286 elongation rate differed by region and was not uniform. One notable difference  
287 was that the elongation of the duodenum was considerable in their study but  
288 relatively inconspicuous in ours.

289 Kluth et al. (1995, 2003) and Metzger et al. (2011) pointed out the  
290 formation of the duodenal loop, with rapid longitudinal elongation in the early  
291 phase and location beneath the root of the mesentery, as a distinct  
292 embryological process. Snyder and Chaffin (1954) consider the position of the

duodenum important because abnormal rotation of the IL tends to be accompanied by an abnormally positioned duodenum; however, Frazer and Robbins (1915) regard it as not important. For our part, we agree with Kluth et al. and Snyder and Chaffin that the early-stage positioning of the duodenojejunal loop beneath the root of the mesentery is a crucial factor which is related to the position of point S, though it may result not from the active elongation of the duodenum but rather from differential growth.

S and E were defined as the most obvious dorsal inflection points on the 3D image in the present study. In Soffer et al.'s study (2015), using a lateral view of a 3D reconstruction, two inflections concomitant with the change in length of the mesentery were clearly observed at points corresponding to S and E in our study. The point of inflection on the anal side was consistent with the boundary of blood supply between the SMA and inferior mesenteric artery, whereas the point on the oral side was located within the blood supply of the SMA. Thus, Soffers et al. regard the duodenum and proximal jejunum, including the inflection points, as part of the midgut loop, namely the first of four secondary loops of the midgut loop. The first secondary loop (proximal duodeno-jejunal loop) differed from other three loops in that the mesentery was thin at the oral side of the inflection, the elongation speed was not so rapid, and no tertiary loop was formed in that region; further, it was rotated 180 degrees relative to the SMA. These observations were almost consistent with ours, though the region was observed as part of segment PS (duodenum) in our study.

The morphology of the IL was rather simple until CS 18. O'Rahilly and Müller (1987) described the IL as entering the umbilical cord without coiling, a

finding similar to that of our present study. However, after CS 19, the small intestinal portion (segment SC) elongated and formed a highly complex coiled, spiral structure. To describe these movements precisely requires definitions of the observation points and reference landmarks. Many previous studies have not used such definitions, resulting in discrepancies among their descriptions of rotation (Mall, 1898; Frazer and Robbins, 1915; O'Rahilly and Muller, 2001; Cyr et al., 1986; Snyder and Chaffin, 1954; Kim et al., 2003; Dott, 1923; Kluth et al., 1995, 2003; Metzger et al., 2011). The present study defined the observation plane and origin by the proximal part of SMA, leading to clear and reliable observations of the movement of points S and E. On the other hand, the observed position of the cecum in the umbilical cord was still variable. One obvious reason for this was that our observation plane was not defined with reference to the cecum. To analyze the movement of the cecum, it would be best to select an observation plane including the cecum itself; however, anatomical reference points outside the intestinal tract, such as the SMA, umbilical vein and arteries, mesentery, and omphaloenteric duct, will be necessary.

The morphogenesis of looping of the embryonic gut and other tubular organs such as the heart have recently been analyzed from a biophysical point of view using biomechanical and computational models. Savin et al. (2011) showed that the morphogenesis of intestinal looping is driven by the homogeneous and isotropic forces that arise from differences in growth rates between the intestine and the anchoring dorsal mesenteries. Hirashima (2014) showed that axial tubular buckling triggered by cell proliferation drives the

morphogenesis of murine epididymal tubules through mechanical interactions between the developing epithelial tubule and its surrounding tissues. Bayraktar and Maenner (2014) indicate that cardiac looping may be driven by compressive loads resulting from unequal growth of the heart and pericardial cavity. Soffers et al. (2015) presented models explaining midgut looping, suggesting that the primary, secondary, and tertiary loops arise in hierarchical fashion; the primary and secondary loops may be regulated genetically, whereas the tertiary loops are variable, with their morphogenesis likely dependent on biophysical factors.

Our results indicate that rotation is a passive event both at earlier stages (CS 14–16) and at later stages (after CS17) (Fig. 7). The movement of segment SE may be explained as differential growth rather than rotation around the origin (SMA). The early-stage positioning of the duodenojejunal loop beneath the root of the mesentery is a crucial factor, though it may also result not from the active elongation of the duodenum. The morphogenesis of the intestinal tract continues after PUH, including events such as the return of the IL and cecum to the abdominal coelom and the fixation of each region in its appropriate final position. To fully determine the nature of IL rotation and its significance to the morphogenesis of the intestinal tract, future studies should observe IL development up to the end of fixation.

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369

370    **Literature cited**

- 371    Bayraktar M, Maenner J. 2014. Cardiac looping may be driven by compressive  
372    loads resulting from unequal growth of the heart and pericardial cavity.  
373    Observations on a physical model. *Front Physiol* 5:1-15.
- 374    Cyr DR, Mack LA, Schoenecker SA, Patten RM, Shepard TH, Shuman WP,  
375    Moss AA. 1986. Bowel migration in the normal fetus: US detection. *Radiology*  
376    161:119-121.
- 377    Dott NM. 1923. Anomalies of intestinal rotation: their embryological and surgical  
378    aspects, with report of five cases. *Br J Surg* 53: 251–286.
- 379    Frazer JE, Robbins RH. 1915. On the factors concerned in causing rotation of  
380    the intestine in man. *J Anat Physiol* 50: 75–110.
- 381    Hirashima T. 2014. Pattern formation of an epithelial tubule by mechanical  
382    instability during epididymal development. *Cell Reports* 9:866-873.
- 383    Kaigai N, Nako A, Yamada S, Uwabe C, Kose K, Takakuwa T. 2014.  
384    Morphogenesis and three-dimensional movement of the stomach during the  
385    human embryonic period. *Anat Rec (Hoboken)* 297: 791–797.
- 386    Kim WK, Kim H, Ahn DH, Kim MH, Park HW. 2003. Timetable for intestinal  
387    rotation in staged human embryos and fetuses. *Birth Defects Res A Clin Mol*  
388    *Teratol* 67:941-945.
- 389    Kluth D, Jaeschke-Melli S, Fiegel H. 2003. The embryology of gut rotation.  
390    *Semin Pediatr Surg* 12:275-279.
- 391    Kluth D, Kaestner M, Tibboel D, Lambrecht W. 1995. Rotation of the gut: fact or  
392    fantasy? *J Pediatr Surg* 30:448-453.



- 393 Mall FP. 1898. Development of the human intestine and its position in the adult.  
394 Bull Johns Hopkins Hosp 9: 197–208.
- 395 Matsuda Y, Ono S, Otake Y, Handa S, Kose K, Haishi T, Yamada S, Uwabe C,  
396 Shiota K. 2007. Imaging of a large collection of human embryo using a  
397 super-parallel MR microscope. Magn Reson Med Sci 6:139-146.
- 398 Matsuda Y, Utsuzawa S, Kurimoto T, Haishi T, Yamazaki Y, Kose K, Anno I,  
399 Marutani M. 2003. Super-parallel MR microscope. Magn Reson Med  
400 50:183-189.
- 401 Meckel JF. 1817. Blindungsgeschichte des Darmkanals der Säugetiere und  
402 namentlich der Menschen. Deutsche Arch Physiol 3: 1–84.
- 403 Metzger R, Metzger U, Fiegel HC, Kluth D. 2011. Embryology of the midgut.  
404 Semin Pediatr Surg 20:145-151.
- 405 Nishimura H, Takano K, Tanimura T, Yasuda M. 1968. Normal and abnormal  
406 development of human embryos: first report of the analysis of 1,213 intact  
407 embryos. Teratology 1:281-290.
- 408 O'Rahilly R, Müller F. 1987. Developmental stages in human embryos : including  
409 a revision of Streeter's Horizons and a survey of the Carnegie Collection.  
410 Washington, D.C.: Carnegie Institution of Washington.
- 411 O'Rahilly R, Müller F. 2001. Human embryology and teratology. 3rd ed, New  
412 York: Wiley-Liss.
- 413 Pernkopf E. 1925. Die Entwicklung der Form des Magendarmkanales beim  
414 Menschen. II. Teil. 1. Abschnitt. Zschr f Anat Entwickl Gesch 77:1-143.
- 415 Savin T, Kurpios NA, Shyer AE, Florescu P, Liang H, Mahadevan L, Tabin CJ.  
416 2011. On the growth and form of the gut. Nature 476:57-62.

417 Shiota K, Yamada S, Nakatsu-Komatsu T, Uwabe C, Kose K, Matsuda Y, Haishi  
418 T, Mizuta S, Matsuda T. 2007. Visualization of human prenatal development by  
419 magnetic resonance imaging (MRI). *Am J Med Genet A* 143A:3121-3126.

420 Snyder WH Jr, Chaffin L. 1952. An intermediate stage in the return of the  
421 intestines from the umbilical cord; embryo 37 mm. *Anat Rec* 113: 451–457.

422 Snyder WH Jr, Chaffin L. 1954. Embryology and pathology of the intestinal tract:  
423 presentation of forty cases of malrotation. *Ann Surg* 110: 368–380.

424 Soffers JH, Hikspoors JP, Mekonen HK, Koehler SE, Lamers WH. 2015. The  
425 growth pattern of the human intestine and its mesentery. *BMC Dev Biol* 15:31.

426 Yamada S, Uwabe C, Nakatsu-Komatsu T, Minekura Y, Iwakura M, Motoki T,  
427 Nishimiya K, Iiyama M, Kakusho K, Minoh M, Mizuta S, Matsuda T, Matsuda Y,  
428 Haishi T, Kose K, Fujii S, Shiota K. 2006. Graphic and movie illustrations of  
429 human prenatal development and their application to embryological education  
430 based on the human embryo specimens in the Kyoto collection. *Dev Dyn*  
431 235:468-477.

## Figure legends

**Figure 1.** The reference axis and anatomical landmarks used in the present study. (a) Illustration showing the reference axis and anatomical landmarks on the intestinal loop. The red broken line represents the border between the abdominal coelom and extraembryonic coelom in the umbilical cord, and the blue broken line connects the two reference points on the superior mesenteric artery (SMA), Z1 and Z2, which were used to define the z-axis and origin. P is the point located above the neural tube and vertebral column near the pyloric antrum, which was used to determine the median plane and y-axis. St is the stomach. (b) Reconstructed intestinal tract with anatomical references and 3D orthogonal coordinate system. (c) Measure for height of the PUH. (d) Orthogonal coordinate system. The SMA is the origin. P, pyloric antrum; S, start of intestinal loop; E, end of intestinal loop; C, cecum; T, tip of the intestinal loop; B, area on the colon crossing the border between the abdominal coelom and extraembryonic coelom;  $\angle Ro$ , angle between median plane and segment SE;  $\angle s$ , angle between S and median plane;  $\angle e$ , angle between E and median plane

**Figure 2.** Representative 3D reconstructions of intestinal tract between CS 14 and CS 23. (a) Ventral views (CS 14, 15). (b) Representative images at CS 16: mid-sagittal section of MR image (i), lateral view with silhouette of embryo (ii), and ventral (iii) and lateral (iv) views of a reconstruction of the intestinal tract alone. (c) Representative images at CS 18: mid-sagittal section of MR image (i);

lateral (ii) and ventral (iii) views with silhouette of embryo. The afferent part of the intestinal loop runs on the right of the SMA, with the efferent part and cecum on the left. The SMA runs parallel to the straight parts of the intestinal loop. (d)

Lateral view of embryos between CS 19 and CS 23.

St, stomach; C, cecum, Va, vermiform appendix; Oe, omphaloenteric duct; red broken line, border between abdominal coelom and extraembryonic coelom in umbilical cord; white line, z-axis. T = height of the PUH (mm); scale bar = 1 mm

**Figure 3.** Elongation of intestinal tract between CS 14 and CS 23. The crown-rump length (CRL) is indicated in line graphs for comparison. Data for regional growth are provided only for CS 16 onward, because points C and S were not evident at CS 14.

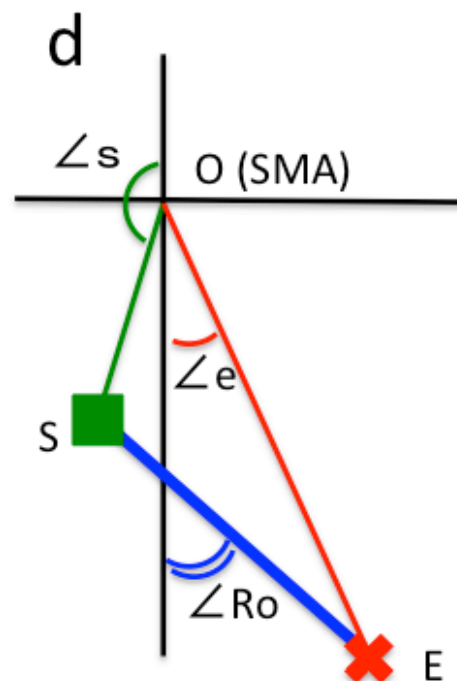
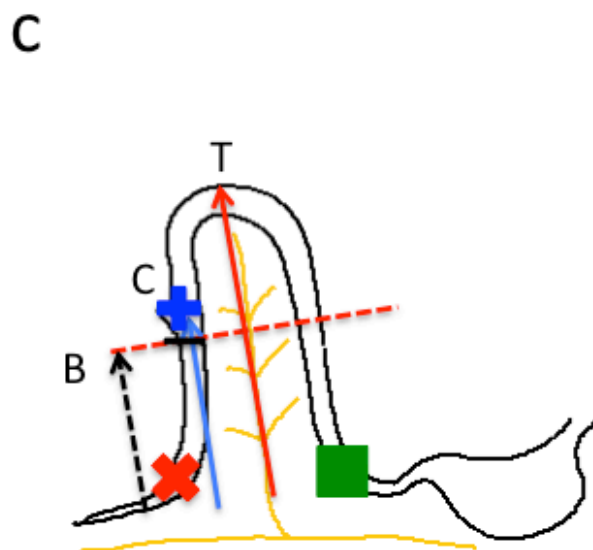
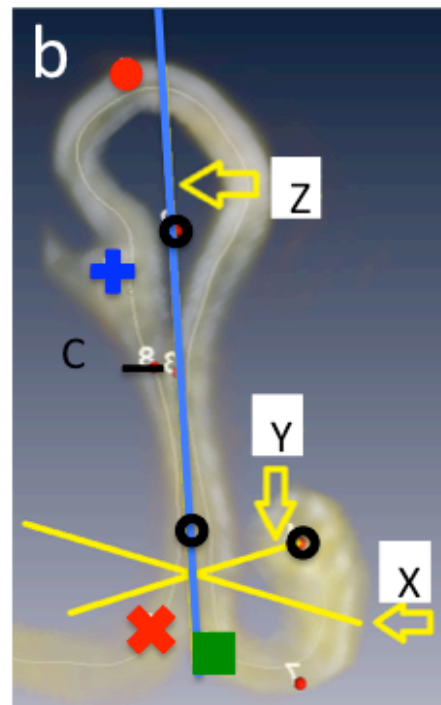
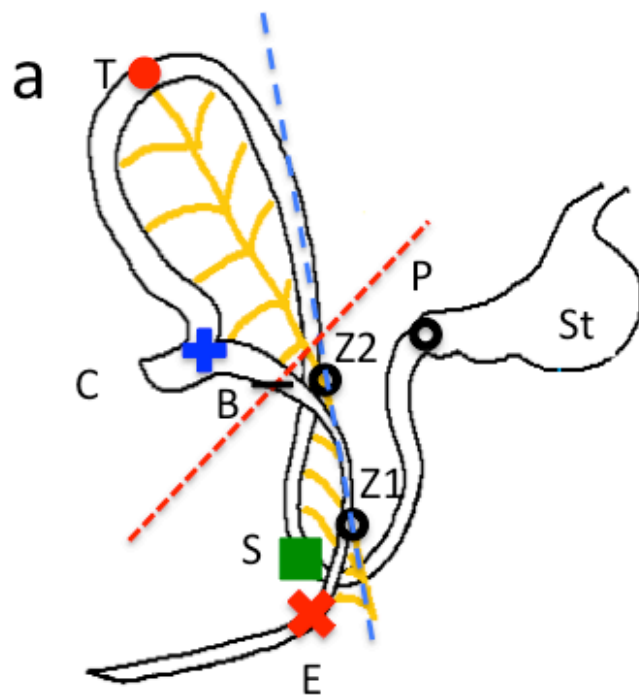
**Figure 4.** Changes in height of the intestinal tract between CS 14 and CS 23, indicating the degree of PUH. The crown-rump length (CRL) and height of the border between the abdominal coelom and the extraembryonic coelom in the umbilical cord are indicated as broken lines for comparison.

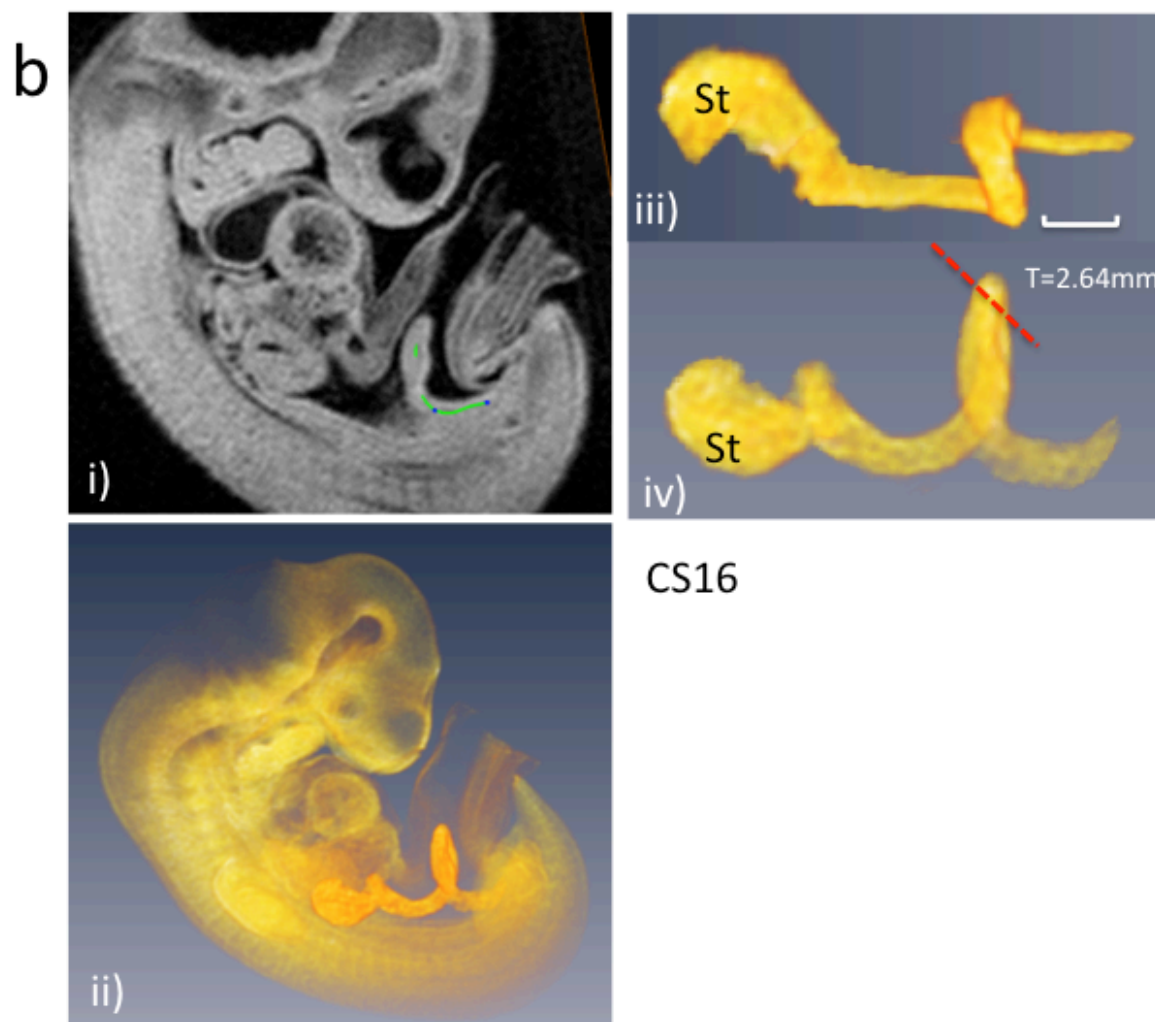
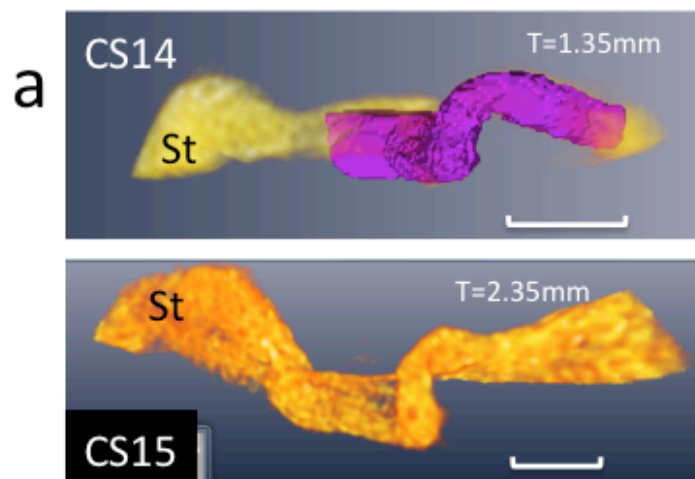
**Figure 5.** Movement of the anatomical landmarks: points S and E (a) and point C (b). The line following the straight portion of the SMA was defined as the z-axis. The points were projected to the xy plane near the proximal part of the SMA, corresponding to the dorsal wall of the abdominal coelom.

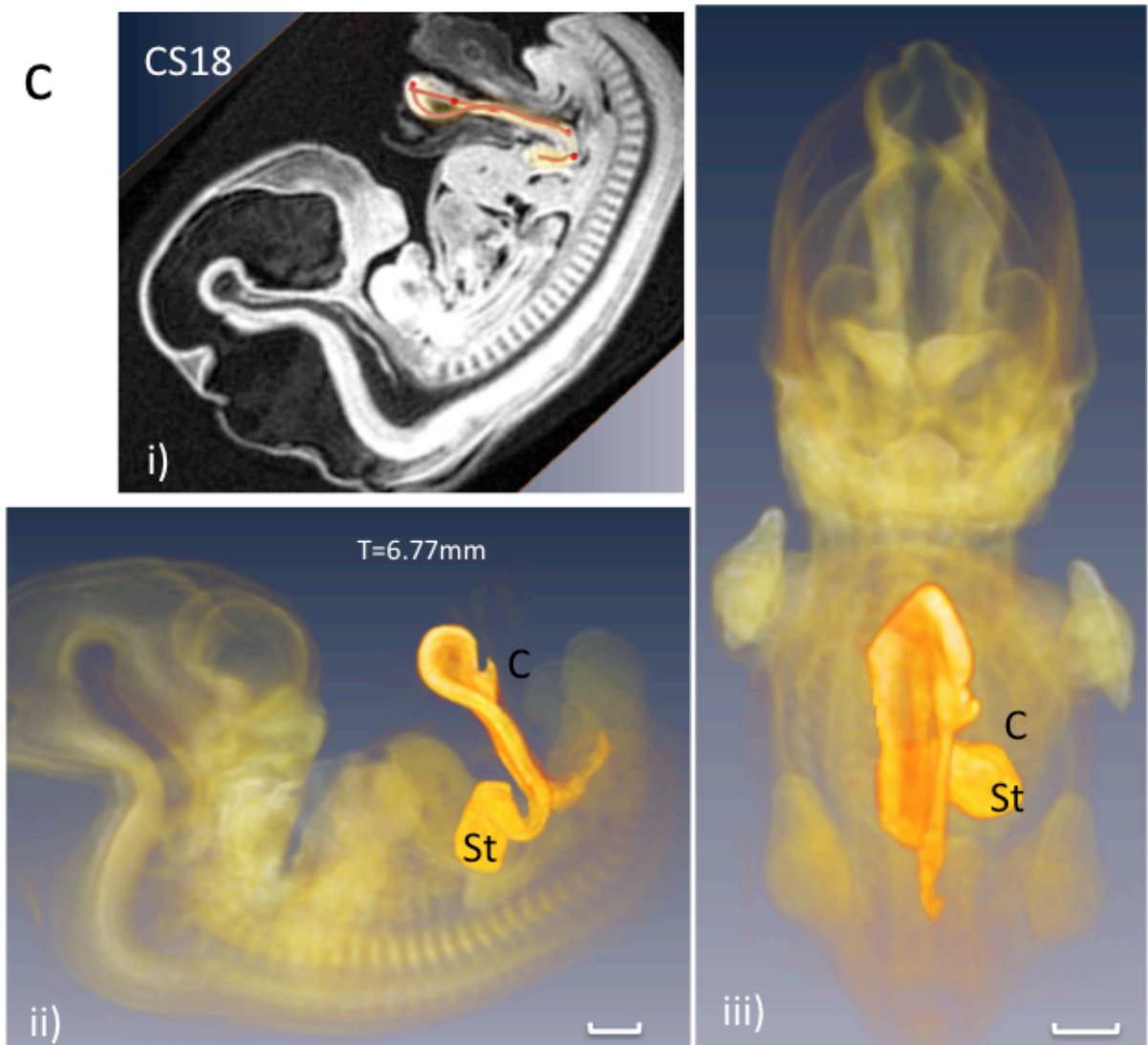
478 **Figure 6.** Relationships of points S and E with the origin (SMA). The angles from  
479 each anatomical landmark to the median plane were plotted during development.  
480  $\angle Ro$ , angle between median plane and segment SE;  $\angle s$ , angle between S and  
481 median plane;  $\angle e$ , angle between E and median plane.

482

483 **Figure 7.** Illustrations showing the movement (viewed ventrally) of the start (S)  
484 and end (E) of the intestinal loop and their positions in relation to the proximal  
485 part of the SMA, designated as the origin (O). (a) Before rotation. (b) Expected  
486 movement according to the classical en bloc model of 90-degree  
487 counterclockwise rotation around SMA as origin. (c) Movement observed  
488 between CS 14 and CS 16. (d) Movement observed from CS 14 to CS 23 (end  
489 of PUH stage).

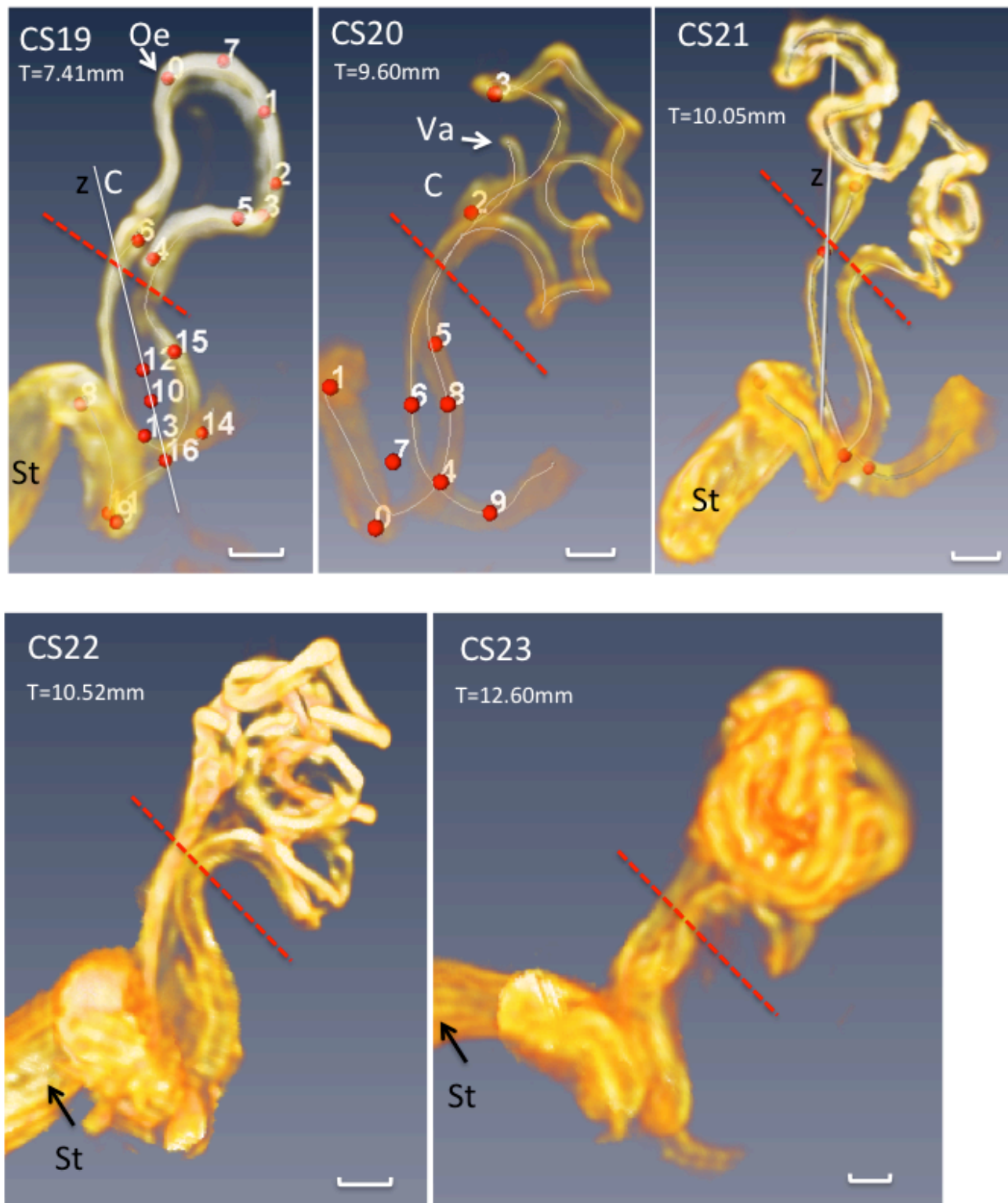


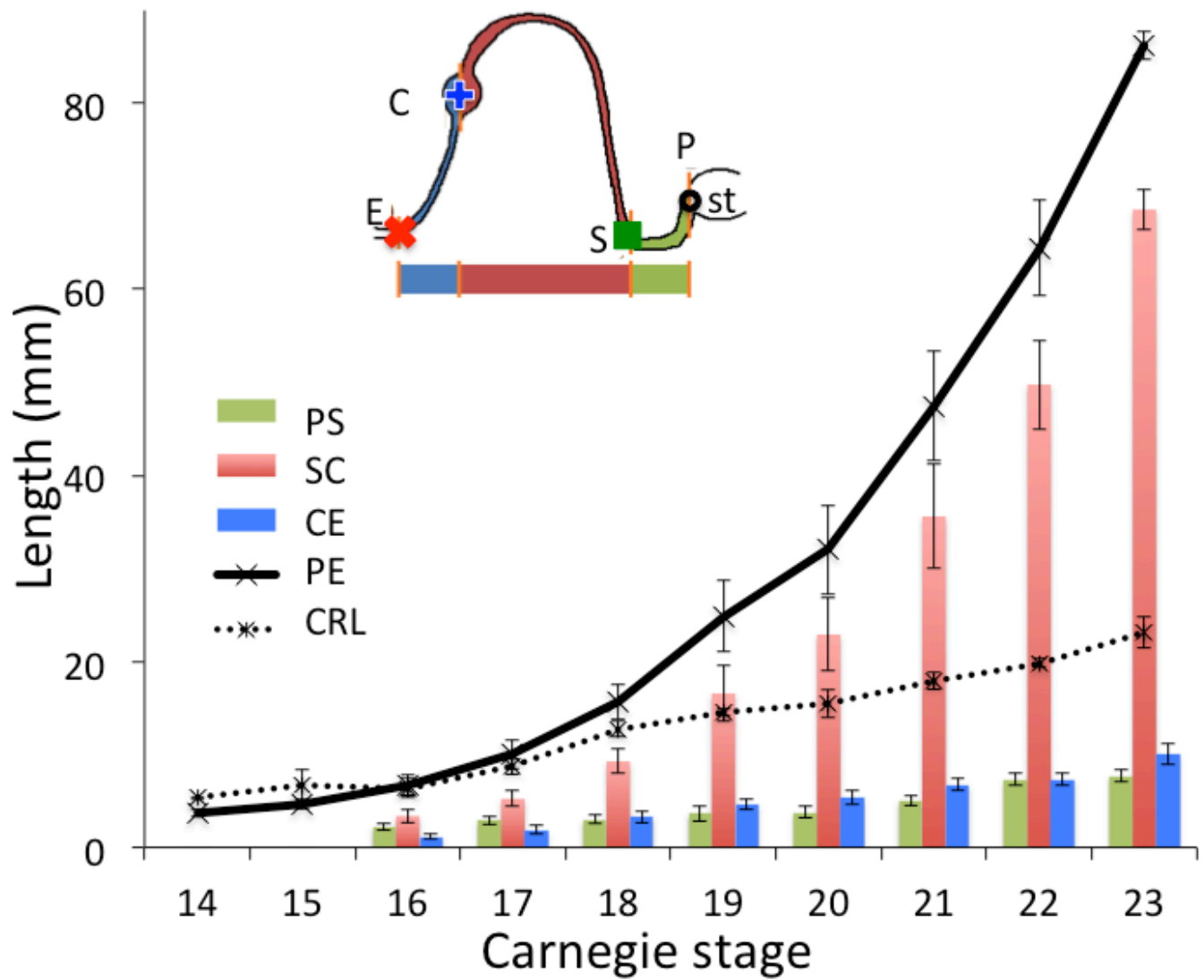


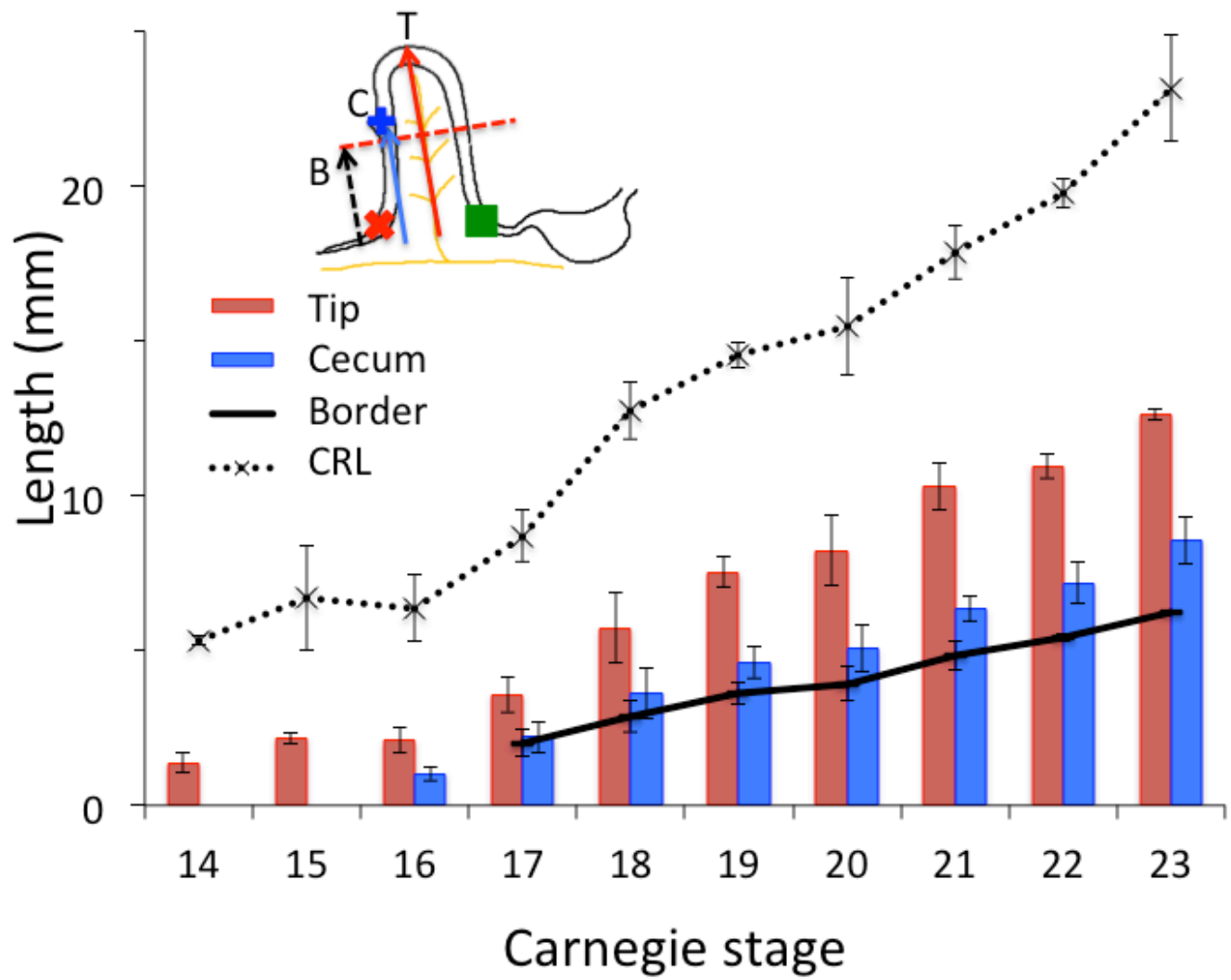


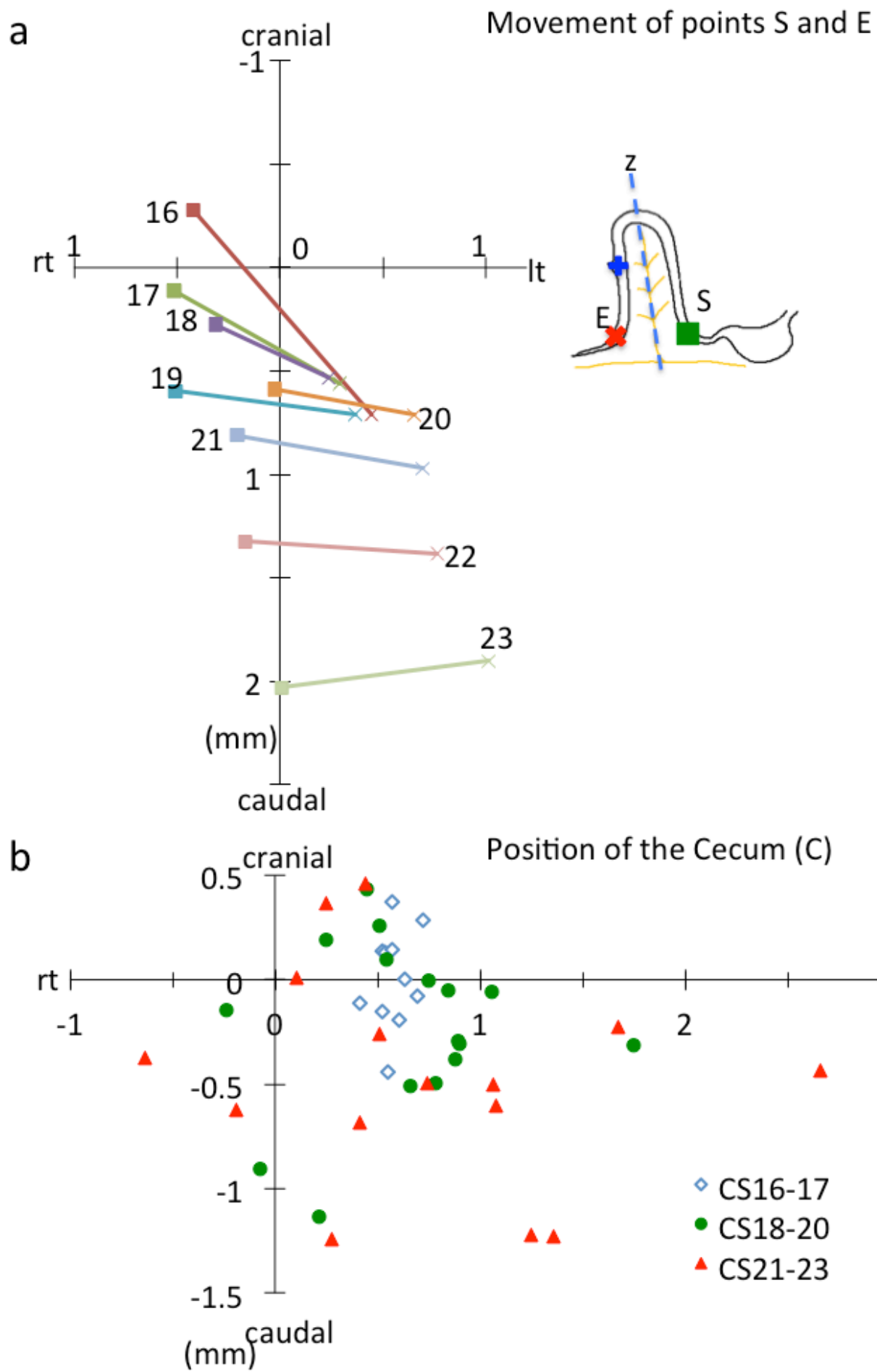


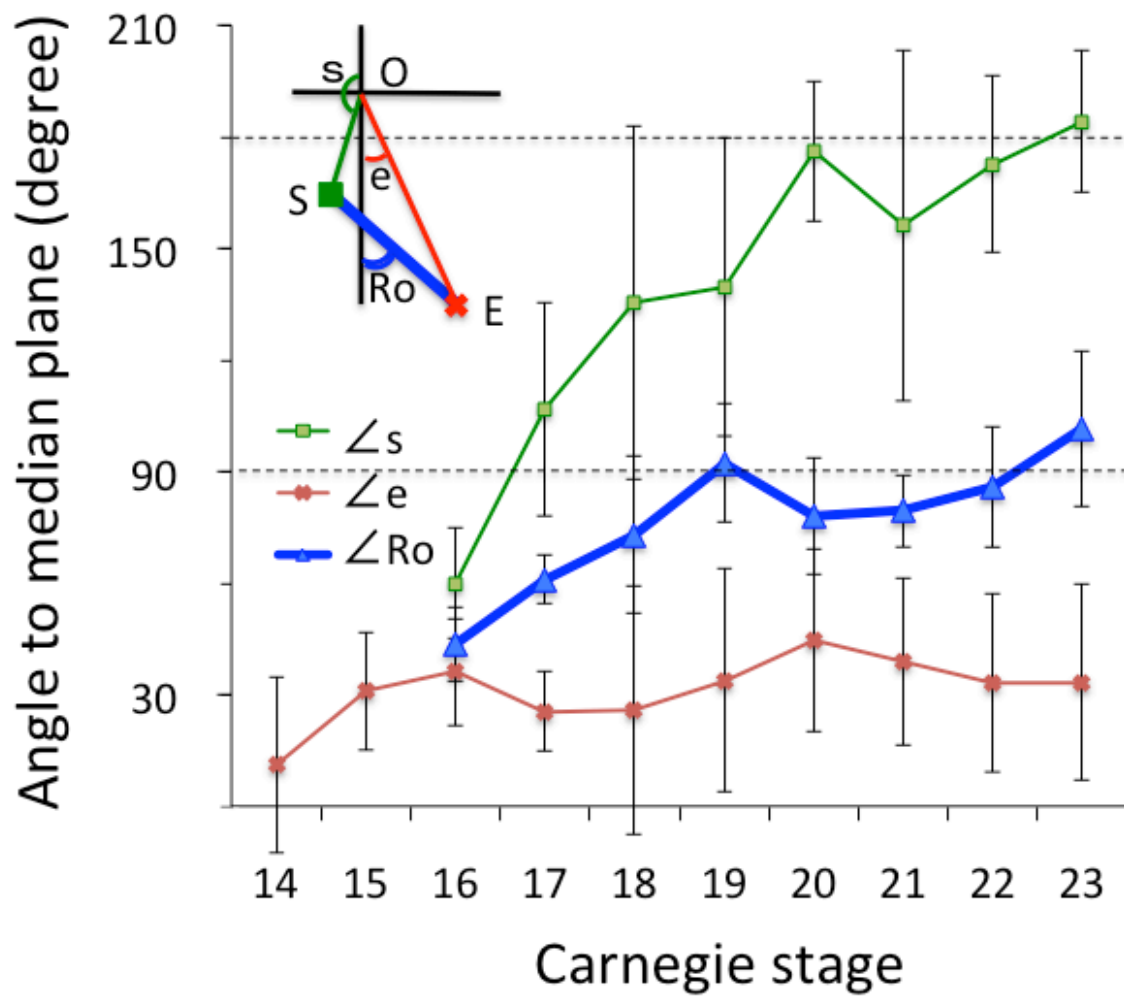
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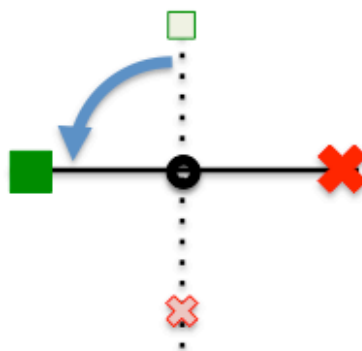




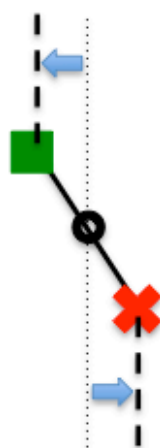
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